INFECTION AND IMMUNITY, Sept. 1995, p. 3502–3506 Vol. 63, No. 9
0019-9567/95/$04.00+0

Entry and Survival of *Leishmania amazonensis* Amastigotes within Phagolysosome-like Vacuoles That Shelter *Coxiella burnetii* in Chinese Hamster Ovary Cells

PATRICIA S. T. VERAS,[1]† CATHERINE MOULIA,[1] CHARLES DAUGUET,[2] CECILE T. TUNIS,[1] MONIQUE THIBON,[3] AND MICHEL RABINOVITCH[1]*

Unite d'Immunoparasitologie, Centre National de la Recherche Scientifique, URA 361,[1] and Unite d'Oncologie Virale[2] and Laboratoire des Rickettsiales et Chlamydiales,[3] Institut Pasteur, 75724 Paris, France

Received 17 January 1995/Returned for modification 15 March 1995/Accepted 2 June 1995

***Coxiella burnetii*, a rickettsia, and *Leishmania amazonensis*, a protozoan flagellate, lodge in their host cells within large phagolysosome-like vacuoles. In the present study, *C. burnetii*-infected Vero or CHO cells were superinfected with *L. amazonensis* amastigotes to determine if these parasites can home to and survive within heterologous vacuoles. Six hours after superinfection, *Leishmania* amastigotes were located almost exclusively within large *Coxiella*-containing vacuoles. Thereafter, the numbers of parasites in the vacuoles increased at the same rate as those in cells infected with *L. amazonensis* alone. Furthermore, in cultures shifted to 25°C, some of the amastigotes transformed into promastigote-like forms that moved their flagella within the adoptive vacuoles. Thus, *L. amazonensis* amastigotes not only entered *Coxiella* vacuoles, most likely by fusion of donor and recipient vacuoles, but temporarily survived, differentiated, and replicated therein. This appears to be the first account of the temporary cohabitation of two living pathogens within the same vacuole in a mammalian cell.**

Coxiella burnetii, the agent of Q fever, and *Leishmania amazonensis*, an agent of cutaneous leishmaniasis, survive and multiply within large phagolysosome-like vacuoles after their phagocytosis by macrophages or other cells (2, 6, 7). These vacuoles are acidified, contain acid hydrolases, and fuse with lysosomes that contain endocytosed colloidal or macromolecular markers such as thorium dioxide, dextrans, or horseradish peroxidase (1, 5, 8, 17, 18, 24). In harmony with their intracellular habitat, *C. burnetii* and *L. amazonensis* are acidophiles, i.e., they survive within the acidic environment of the phagolysosome, exchanging passively acquired protons for needed substrates with the help of plasma membrane ATPases (15, 28).

We have previously shown that zymosan particles or latex beads phagocytized by infected Chinese hamster ovary (CHO) cells are transferred with high efficiency to the lumen of vacuoles that shelter *C. burnetii*. Such transfer most likely takes place by the fusion of donor vacuoles containing the particles and *Coxiella*-rich recipient vacuoles (29).

Given the ease with which relatively large particles can enter *Coxiella* vacuoles, we superinfected *C. burnetii*-infected Vero or CHO cells with *L. amazonensis* amastigotes to determine if the parasites could be targeted to and survive within *C. burnetii* vacuoles. Since the two organisms are easily distinguished by light microscopy, it was possible to enumerate the amastigotes in the heterologous vacuoles. We show here that *L. amazonensis* can home to and, at least temporarily, survive, replicate, and differentiate within adoptive *Coxiella* vacuoles.

(Preliminary results of this work have been presented previously [23]).

* Corresponding author. Mailing address: Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Ave., New York, NY 10021-6399. Phone: (212) 327-8103. Fax: (212) 327-8875. Electronic mail address: rabinom@rockvax.rockefeller.edu.

† Present address: Servico de Imunologia, HUPES, Universidade Federal da Bahia, 40140 Salvador, Bahia, Brazil.

MATERIALS AND METHODS

Cell culture. CHO cells, provided by Bruno Goud from the Pasteur Institute, were used in most experiments. These cells were grown in T75 flasks in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 g of sodium bicarbonate per liter, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 2 mM glutamine, and 20 μg of gentamicin per ml. Vero cells, used in the early experiments, were similarly cultured in minimal essential medium supplemented with 5% fetal calf serum, 2.2 g of sodium bicarbonate per liter, and 25 mM HEPES. Cells detached with trypsin-EDTA were plated on antibiotic-free media on 12-mm coverslips distributed in 24-well plates and were incubated at 34°C in an air atmosphere.

Organisms. *C. burnetii* phase II (the Nine Mile strain) was propagated in Vero cell cultures and harvested as described previously (29). *Coxiella*-rich suspensions contained about 10^7 infective organisms per ml as judged by the infection of monolayers with serial dilutions of Vero cell lysates. *L. amazonensis* LV79 (strain designation, MPRO/BR/72/M18410) serially transferred in the footpads of female BALB/c mice (Animal Facility, Pasteur Institute) was harvested as described previously (29).

Infection of cells. Vero or CHO cells grown overnight on coverslips were infected with *C. burnetii*, usually by centrifugation of the bacteria over the cell monolayers at an estimated multiplicity of 200 bacteria per cell (29). During the infection and for 4 h afterwards, the cells remained in an air atmosphere in bicarbonate-free medium containing 5% serum and 25 mM HEPES. After the infection, the cultures were washed, added to complete medium containing 5% serum, and placed in the CO_2 incubator. The serum concentration was lowered to reduce the rate of multiplication of the host cells. Two to three days after the infection with *C. burnetii*, the cells were superinfected with three to five *L. amazonensis* amastigotes per cell (29). Separate cultures were infected with *L. amazonensis* alone. Cultures infected with *C. burnetii* or *L. amazonensis* were washed after 4 or 6 h, respectively, the media were changed, and the cells were replaced at 35°C in a 5% CO_2–air atmosphere. At different times, the cultures were rinsed and fixed overnight in 1% glutaraldehyde in phosphate-buffered saline (PBS). In some experiments, this design was modified as follows. To destroy intracellular amastigotes, the cultures were incubated 24 h prior to fixation in medium containing 2 mM L-leucyl-*o*-methyl ester (3). To trigger the transformation of the amastigotes into promastigotes, the cultures, 24 h prior to fixation, were placed in bicarbonate-free, complete medium containing 25 mM HEPES at 25°C in an air atmosphere. Coverslips bearing the cells were inverted over 60% glycerol in water onto standard microscope slides. The preparations were examined by phase-contrast or Nomarski differential-interference microscopy. The cultures were scored for the percentage of infected cells that contained *C. burnetii* or *L. amazonensis* within the same vacuoles as well as for the numbers of amastigotes per cell that colocalized with the bacteria. Cultures infected with *L. amazonensis* alone were also scored for the percentage of infected cells and the numbers of amastigotes per cell.

Electron microscopy. Cultures in 60-mm-diameter dishes were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2, and postfixed in 1% osmium

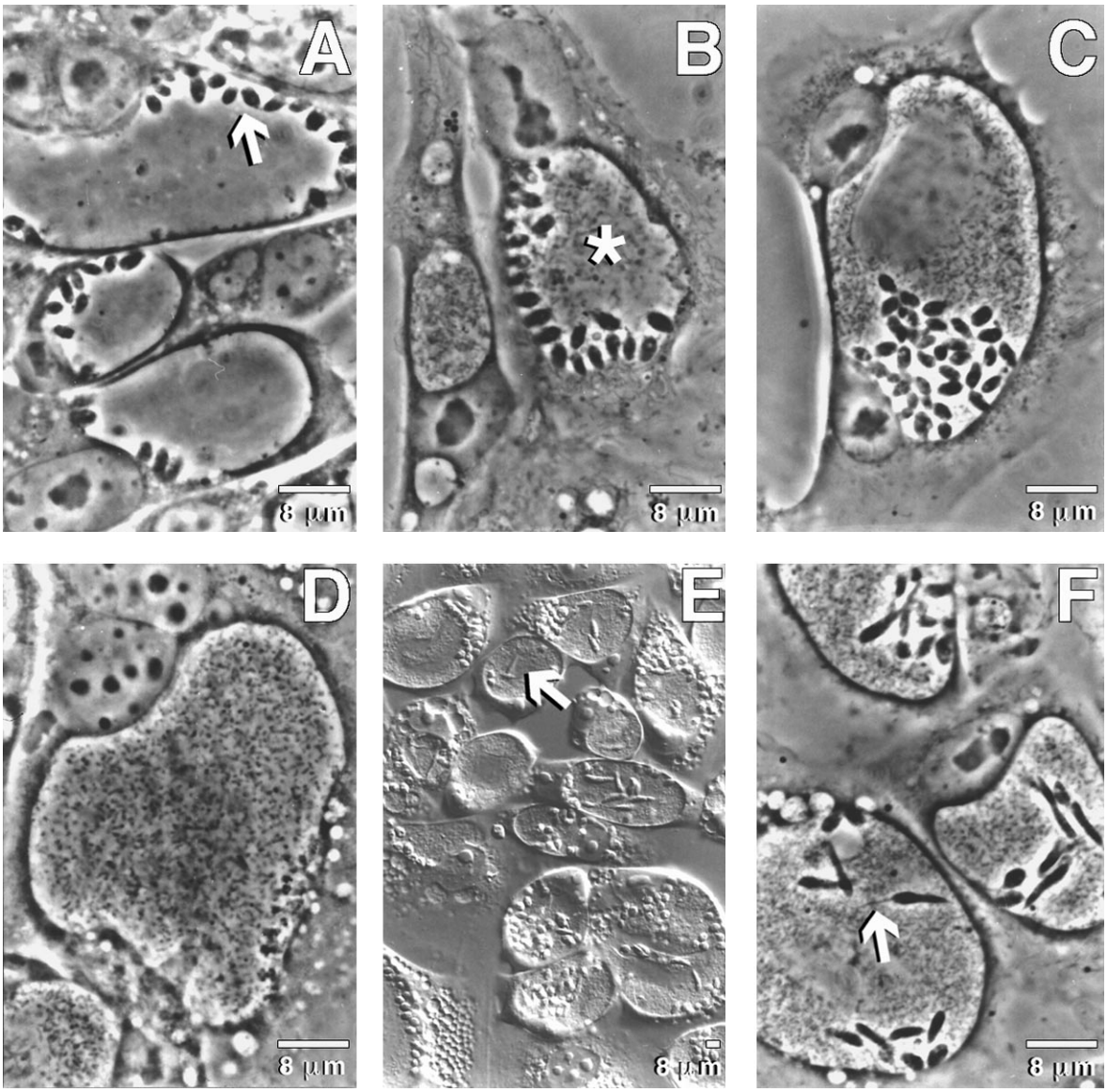

FIG. 1. Colonization by *L. amazonensis* amastigotes of vacuoles induced by *C. burnetii*. All panels are phase-contrast micrographs unless indicated otherwise. (A) CHO cells infected for 24 h with *L. amazonensis* alone. Amastigotes (arrow) are aligned on the inner surface of the PV membrane. (B) Vero cells infected for 3 days with *C. burnetii* and superinfected with *L. amazonensis* for 4 days. *Coxiella* organisms are the small, dense particles in the center of the large vacuole (*). (C) CHO cells infected for 3 days with *C. burnetii* and then for 1 day with *L. amazonensis*. Amastigotes are clustered in the lower part of the vacuole. (D) CHO cells infected for 3 days with *C. burnetii* and for 1 day with *L. amazonensis* and incubated for another 24 h in 2 mM L-leucyl-*o*-methyl ester prior to fixation. Remnants of amastigotes are seen on the right side of the vacuole. (E and F) CHO cells infected for 3 days with *C. burnetii* and superinfected for 2 days with *L. amazonensis* at 34°C and shifted to 25°C prior to fixation. (E) Nomarski micrograph showing numerous amastigotes and a few promastigotes (arrows). *Coxiella* organisms are not clearly shown in this low-magnification photograph. (F) The arrow points to a promastigote flagellum. A few, apparently nontransformed amastigotes can be seen in the cell at the top of the micrograph.

tetroxide. The cells were dehydrated in ethanol and embedded in situ in Glycid-ether Resin 100 (Merck, Darmstadt, Germany). Thin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100 CXII electron microscope.

RESULTS

***L. amazonensis* amastigotes can enter preestablished *C. burnetii* vacuoles.** CHO or Vero cells were infected with *C. burnetii*, and 2 or 3 days later, when large vacuoles containing the bacteria had developed in most cells, the cultures were superinfected with *Leishmania* amastigotes. The cells were fixed at different times of from between 6 h to 6 days after superinfection. Amastigotes were found within *Coxiella*-rich vacuoles of the host cells in 11 separate experiments. In eight experiments, the percentage of cells with doubly infected vacuoles and the numbers of amastigotes that colocalized with *C. burnetii* per cell were also estimated at one or more time points after superinfection. One or two days after the infection of Vero or CHO cells with *L. amazonensis* amastigotes alone, most of the parasites were aligned on the inner surface of a few large, otherwise phase-lucent parasitophorous vacuoles (PVs) (Fig. 1A). The origin of these vacuoles is not understood, but time-lapse cinemicrographic observations of macrophages infected with *L. amazonensis* indicated that small PVs only rarely fuse

with each other (31). Cells infected with *C. burnetii* alone generally contained one or two large vacuoles that occupied a large fraction of the cytoplasm and were closely apposed to the cell nucleus. As was observed earlier (27), these large vacuoles appear to develop by fusion of small vacuoles that contain one or a few bacteria. In living cells examined by phase-contrast or interference microscopy, *C. burnetii* was seen as small rounded or elongated bodies dispersed within the vacuoles. Figure 1B shows a *Coxiella*-infected cell superinfected with *L. amazonensis* and fixed 4 days after superinfection. The distribution of the parasites mimics their localization in cells infected only with *L. amazonensis*; more frequently, however, *L. amazonensis* was eccentrically placed and often clumped within the *Coxiella* vacuoles (Fig. 1C). Vacuoles containing only *L. amazonensis* were rarely observed in doubly infected cells.

Transmission electron microscopy. Confirmation that *Leishmania* amastigotes were localized to *Coxiella* vacuoles was provided by transmission electron microscopy. Figure 2A shows a cell from a culture infected for 5 days with *C. burnetii* alone, and Fig. 2B displays well-preserved amastigotes surrounded by *C. burnetii* in a parallel culture fixed 2 days after superinfection with *L. amazonensis*.

Fixed *L. amazonensis* also enters *Coxiella* vacuoles. To determine if the parasites needed to be alive to be transferred to *Coxiella* vacuoles, amastigotes were fixed overnight at 4°C in 1% glutaraldehyde in PBS, washed, counted, and added overnight to *Coxiella*-infected CHO cultures. Fixed amastigotes were easily found in the *Coxiella*-rich vacuoles (data not shown), indicating that metabolic activity by the parasites was not required for their transfer to the heterologous vacuoles.

***L. amazonensis* amastigotes increase in numbers within *Coxiella* vacuoles.** Figure 3A shows that 6 h after superinfection, amastigotes were present within the *Coxiella*-rich vacuoles of most cells. Thereafter, whereas the percentage of CHO cells that contained doubly infected vacuoles fell with time after superinfection, the average number of amastigotes per vacuole increased severalfold. The reduction in the proportion of infected cells probably reflects faster multiplication of uninfected cells, whereas the increased numbers of parasites per vacuole is most likely due to multiplication of the amastigotes. Figure 3B displays the results of parallel cultures infected with *L. amazonensis* alone. In these cultures, the percentage of *Leishmania*-infected cells also fell with time and the numbers of amastigotes within the *Leishmania* PVs increased. It should be noted that 6 h after infection with *L. amazonensis* alone, PVs containing amastigotes were quite small and vacuolar space was rarely seen in these vacuoles. Furthermore, 24 h after *Coxiella*-infected cells were challenged with amastigotes, only rare pure *Leishmania* vacuoles (i.e., devoid of *C. burnetii*) were found. It is thus likely that most doubly infected vacuoles were, at first, occupied by *C. burnetii*. After 6 days of superinfection, the high cell density of the cultures made quantitative studies unreliable.

Amastigotes within *Coxiella* vacuoles can be killed by L-leucyl-*O*-methyl ester. The viability of the parasites was also tested by incubation of doubly or singly infected cultures with 2.0 mM L-leucyl-*o*-methyl ester. Nearly all amastigotes within *Coxiella* vacuoles were destroyed by 24 h of exposure of the cultures to the leucyl ester (Fig. 1D). Parasites within *Leishmania* vacuoles were similarly sensitive to the compound (data not shown).

Amastigotes can transform into promastigotes within *Coxiella* vacuoles. A small proportion of amastigotes transformed into L-leucyl-*o*-methyl ester-resistant promastigotes when cultures were shifted for 24 h to 25°C (Fig. 1E and F). The rapid movement of the promastigote flagella displaced the bacteria within the vacuoles and was documented by real-time video. The presence of division forms indicated that at least some of the promastigotes multiplied within the *Coxiella*-rich vacuole; furthermore, promastigotes recovered an amastigote-like morphology when the cultures, pulsed at 25°C, were again shifted to 35°C (data not shown). However, the percentage of vacuoles in which promastigotes could be found after the temperature was shifted down was highest 6 h after superinfection and fell substantially at 4 days, indicating that the parasite's potential for differentiation was progressively restricted with time of residence within the *Coxiella*-rich vacuole.

DISCUSSION

This is, to our knowledge, the first report of in vitro temporary colonization by one pathogen (*L. amazonensis*) of the vacuoles occupied by another (*C. burnetii*) in a mammalian cell. The viability of the amastigotes was inferred from their expanding numbers within the adoptive vacuoles, sensitivity to L-leucyl-*O*-methyl ester, and transformation of some of the parasites into flagellated forms at 25°C.

Coinfection of cells with two viruses or virus strains has been frequently reported in the literature. In fewer instances, cells have been doubly infected with a virus and a bacterium or a protozoan (14, 20, 25, 32). Coinfection of cells with two strains of the same organism has also been reported (26). However, there are few reports of mammalian cells being doubly infected with two different prokaryotic or eukaryotic pathogens (9, 19). In one study, macrophages were infected with *Toxoplasma gondii*, a parasite sheltered in atypical fusion-resistant vacuoles (16), to determine if the infection interferes with the fusion of the host cell lysosomes with endocytic vacuoles containing *Trypanosoma cruzi* epimastigotes (4, 19). The fates of the vacuoles were similar in singly and doubly infected cells. In the course of studies of the effect of tumor necrosis factor or gamma interferon on intracellular infection (9), macrophages were coinfected with *T. gondii* and *Mycobacterium avium*, which also survives in vacuoles that resist fusion with lysosomes (10, 13, 33). The authors noted that "phagosomes containing both agents were not common" (9).

In the present experiments, cells were doubly infected with organisms normally lodged in phagolysosome-like vacuoles. Our results documented the ease with which live or killed *L. amazonensis* amastigotes could enter preformed *C. burnetii* vacuoles. To determine if *C. burnetii* could also home to *Leishmania* vacuoles, reciprocal experiments in which cells were infected first with *L. amazonensis* and then with *C. burnetii* were also performed. The results of these experiments were inconclusive, for it was difficult to identify and enumerate a few bacteria within *Leishmania* PVs at early times after superinfection with *C. burnetii*. By 24 h, however, *C. burnetii* was present within *Leishmania* vacuoles (data not shown).

Although direct evidence is difficult to obtain, the transfer of *L. amazonensis* to *Coxiella* vacuoles most likely occurred by the fusion of recipient vacuoles containing the bacteria and donor vacuoles containing the parasites. In another system, namely the transfer of zymosan particles to *Leishmania* PVs in macrophages (30), time-lapse cinemicrographic observations revealed that target vacuoles enlarged as transfer occurred, suggesting that the membranes of the donor vacuoles were incorporated, presumably by fusion, into the PV membrane (31a). However, the stage of maturation of the fusing phagocytic vacuoles has not been defined in the *Coxiella* model. As was indicated in the introduction, *Coxiella* vacuoles are acidified, display acid phosphatase activity, and fuse with vesicles containing endocytosed markers (1, 6, 7, 18). These features

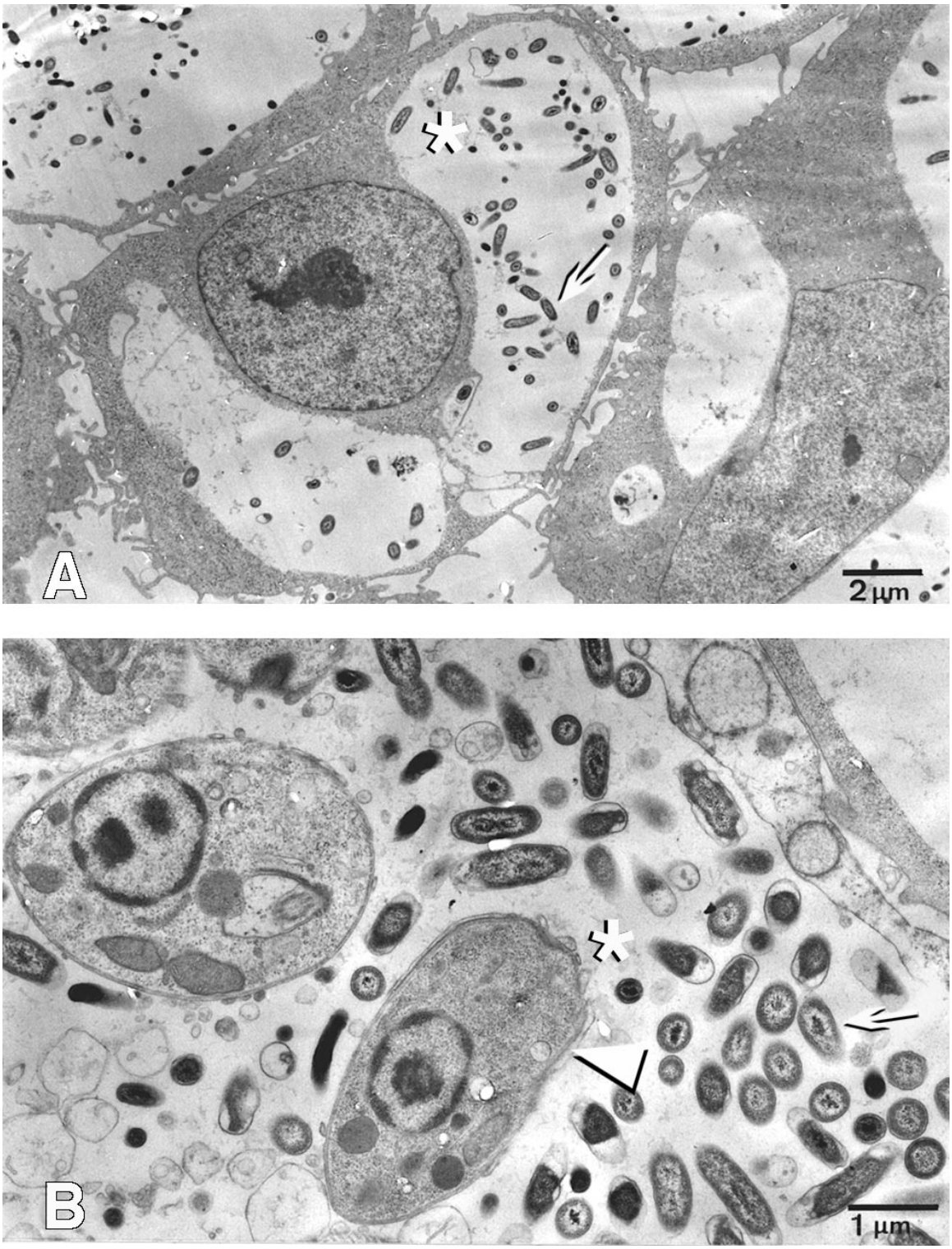

FIG. 2. Ultrastructure of CHO cells infected for 5 days with *C. burnetii* alone (A) and similar cultures infected for 3 days with *C. burnetii*, superinfected with *L. amazonensis*, and fixed 2 days later (B). Asterisks are placed in the lumen of the *Coxiella*-containing vacuoles. Bacteria are indicated by arrows, and the open arrowhead in panel B points to a well-preserved amastigote.

are usually associated with phagolysosomes. However, the presence of markers of earlier endocytic compartments, e.g., GTP-binding Rab proteins or cation-independent mannose phosphate receptors, in the membranes of *Coxiella* vacuoles needs to be evaluated (12). More information on *L. amazonensis* PVs is available. These vacuoles display lysosomal glycoprotein and Rab7 reactivity (17), with the latter being associated with a prelysosomal compartment (12).

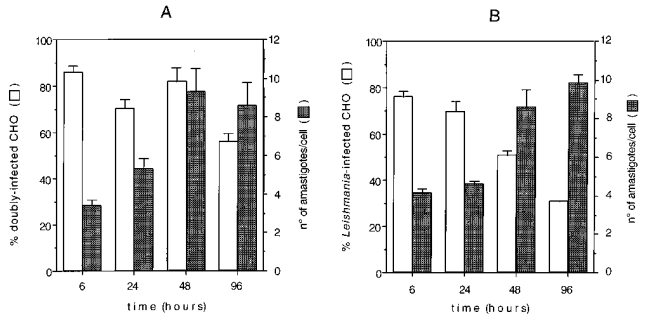

FIG. 3. (A) CHO cells were infected for 3 days with *C. burnetii* and superinfected with *L. amazonensis* for the times shown. The error bars indicate the standard errors of the means for triplicate or quadruplicate coverslips. (B) CHO cells were infected with *L. amazonensis* alone and fixed at the times indicated. Error bars indicate standard errors of the means.

The underlying assumption of the present studies is that cells coinfected with prokaryotic and/or eukaryotic organisms may be useful in the search for pathogen- or cell-derived signals that regulate the fusion of pathogen-containing vacuoles with other endocytic or phagocytic vesicles. It remains to be seen if *Coxiella* vacuoles will fuse with vacuoles that are not typical phagolysosomes, for example, those that shelter *Chlamydia trachomatis*, *T. gondii*, or certain mycobacteria, organisms that inhibit acidification and block the fusion of their vacuoles with other phagocytic vesicles (10, 11, 13, 16, 21, 22, 33). If one or more of these heterologous organisms can enter the *Coxiella* vacuole, then the model proposed here will, in addition, test the ability of the pathogens to survive in a phagolysosomal-like, acidified environment.

ACKNOWLEDGMENTS

This work was supported by Institut Pasteur, CNRS, and INSERM. Patricia S. T. Veras was the recipient of a predoctoral fellowship from CNPq, Brasilia, D.F., Brazil.

We are indebted to F. Gardy and V. Zeitoun for printing the light micrographs and to G. Kaplan and R. Steinman for critically reading the manuscript.

REFERENCES

1. **Akporiaye, E. T., J. D. Rowatt, A. A. Aragon, and O. G. Baca.** 1983. Lysosomal response of a murine macrophage-like cell line persistently infected with *Coxiella burnetii*. Infect. Immun. **40:**1155–1162.
2. **Alexander, J., and D. G. Russell.** 1992. The interaction of *Leishmania* species with macrophages. Adv. Parasitol. **31:**175–254.
3. **Alfieri, S. C., C. Ramazeilles, V. Zilberfarb, I. Galpin, S. E. Norman, and M. Rabinovitch.** 1988. Proteinase inhibitors protect *Leishmania amazonensis* amastigotes from destruction by amino acid esters. Mol. Biochem. Parasitol. **29:**191–201.
4. **Andrews, N. W.** 1995. Lysosome recruitment during host cell invasion by *Trypanosoma cruzi*. Trends Cell Biol. **5:**133–137.
5. **Antoine, J.-C., E. Prina, C. Jouanne, and P. Bongrand.** 1990. Parasitophorous vacuoles of *Leishmania amazonensis*-infected macrophages maintain an acidic pH. Infect. Immun. **58:**779–787.
6. **Baca, O. G., Y. P. Li, and H. Kumar.** 1994. Survival of the Q fever agent *Coxiella burnetii* in the phagolysosome. Trends Microbiol. **2:**476–480.
7. **Baca, O. G., and D. Paretsky.** 1983. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. Microbiol. Rev. **47:**127–149.
8. **Barbieri, C. L., K. Brown, and M. Rabinovitch.** 1985. Depletion of secondary lysosomes in mouse macrophages infected with *Leishmania mexicana amazonensis*; a cytochemical study. Z. Parasitenkd. **71:**159–168.
9. **Black, C. M., L. E. M. Bermudez, L. S. Young, and J. S. Remington.** 1990. Co-infection of macrophages modulates interferon gamma and tumor necrosis factor-induced activation against intracellular pathogens. J. Exp. Med. **172:**977–980.
10. **Clemens, D. A., and M. A. Hurwitz.** 1995. Characterization of the *Mycobacterium tuberculosis* phagosome and evidence that phagosomal maturation is inhibited. J. Exp. Med. **181:**257–270.
11. **de Chastellier, C., C. Frehel, C. Offredo, and E. Skamene.** 1993. Implication of phagosome-lysosome fusion in restriction of *Mycobacterium avium* growth in bone marrow macrophages from genetically resistant mice. Infect. Immun. **61:**3775–3784.
12. **Desjardins, M., L. A. Huber, R. G. Parton, and G. Griffiths.** 1994. Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J. Cell Biol. **124:**677–688.
13. **Falkow, S., R. I. Isberg, and D. A. Portnoy.** 1992. The interaction of bacteria with mammalian cells. Annu. Rev. Cell Biol. **8:**333–363.
14. **Gelderman, A. H., P. M. Grimley, and A. S. Rabson.** 1968. *Toxoplasma gondii* and cytomegalous virus: mixed infection by a parasite and a virus. Science **160:**1130–1132.
15. **Glaser, T. A., and A. J. Mukkada.** 1992. Proline transport in *Leishmania donovani* amastigotes: dependence on pH gradients and membrane potential. Mol. Biochem. Parasitol. **51:**1–8.
16. **Joiner, K. A.** 1994. Vacuolar membranes surrounding intracellular pathogens: where do they come from and what do they do? Infect. Agents Dis. **2:**215–219.
17. **Lang, T., C. de Chastellier, C. Frehel, R. Hellio, P. Metezeau, S. S. Leao, and J. C. Antoine.** 1994. Distribution of MHC class I and MHC class II molecules in macrophages infected with *Leishmania amazonensis*. J. Cell Sci. **107:**69–82.
18. **Maurin, M., A. M. Benoliel, P. Bongrand, and D. Raoult.** 1992. Phagolysosomes of *Coxiella burnetii*-infected cell lines maintain an acidic pH during persistent infection. Infect. Immun. **60:**5013–5016.
19. **Meirelles, M. N., and W. de Souza.** 1983. Interaction of lysosomes with endocytic vacuoles in macrophages simultaneously infected with *Trypanosoma cruzi* and *Toxoplasma gondii*. J. Submicrosc. Cytol. **15:**889–896.
20. **Meylan, P. R. A., J. R. Munis, D. D. Rochman, and R. S. Kornbluth.** 1992. Concurrent human immunodeficiency virus and mycobacterial infection of macrophages in vitro does not reveal any reciprocal effect. J. Infect. Dis. **165:**80–86.
21. **Moulder, J. W.** 1985. Comparative biology of intracellular parasitism. Microbiol. Rev. **49:**298–337.
22. **Moulder, J. W.** 1991. Interaction of chlamydiae and host cells in vitro. Microbiol. Rev. **55:**143–190.
23. **Rabinovitch, M., C. Moulia, P. S. T. Veras, C. Thibaut, V. Villiers, and M. Thibon.** 1993. Peaceful cohabitation of *Leishmania amazonensis* amastigotes and the bacterium *Coxiella burnetii* within the same phagolysosomes of doubly infected Vero cells. Mem. Inst. Oswaldo Cruz Rio J. **88**(Suppl.)**:**116 (Abstract).
24. **Rabinovitch, M., G. Topper, P. Cristello, and A. Rich.** 1985. Receptor-mediated entry of peroxidases into the parasitophorous vacuoles of macrophages infected with *Leishmania mexicana amazonensis*. J. Leukocyte Biol. **37:**247–261.
25. **Reed, S. G., J. S. da Silva, J. L. Ho, J. K. Koechler, D. M. Russom, D. A. Pihl, and R. W. Coombs.** 1992. Cytokine activation of human macrophages infected with HIV1 to inhibit intracellular protozoa. J. Acquired Immune Defic. Syndr. **5:**666–675.
26. **Ridderhof, J. C., and R. C. Barnes.** 1989. Fusion of inclusions following superinfection of HeLa cells by two serovars of *Chlamydia trachomatis*. Infect. Immun. **57:**3189–3193.
27. **Roberts, A. N., and C. M. Downs.** 1959. Study on the growth of *Coxiella burnetii* in the L strain mouse fibroblast and the chick fibroblast. J. Bacteriol. **77:**194–204.
28. **Thompson, H. A., and J. C. Williams.** 1991. pH and membrane physiology in *Coxiella burnetii*, p. 117–129. *In* J. C. Williams and H. A. Thompson (ed.), Q fever: the biology of *Coxiella burnetii*. CRC Press, Boca Raton, Fla.
29. **Veras, P. S. T., C. de Chastellier, M. F. Moreau, V. Villiers, M. Thibon, D. Mattei, and M. Rabinovitch.** 1994. Fusion between large phagocytic vesicles: targeting of yeast and other particulates to phagolysosomes that shelter the bacterium *Coxiella burnetii* or the protozoan *Leishmania amazonensis* in Chinese hamster ovary cells. J. Cell Sci. **107:**3065–3076.
30. **Veras, P. S. T., C. de Chastellier, and M. Rabinovitch.** 1992. Transfer of zymosan (yeast cell walls) to the parasitophorous vacuoles of macrophages infected with *Leishmania amazonensis*. J. Exp. Med. **176:**639–646.
31. **Veras, P. S. T., M. Rabinovitch, and M. Pouchelet.** Unpublished data.

31a. **Veras, P. S. T., A. Topilko, N. Gouhier, M. F. Moreau, M. Rabinovitch, and M. Pouchelet.** Unpublished data.

32. **Welker, Y., J.-M. Molina, C. Poirot, F. Ferchal, J.-M. Decazes, P. Lagrange, and F. Derouin.** 1993. Interaction between human immunodeficiency virus and *Toxoplasma gondii* replication in dually infected monocytoid cells. Infect. Immun. **61:**1596–1598.
33. **Xu, S., A. Cooper, S. Sturgill-Koszycki, T. van Heyningen, D. Chatterjee, I. Orme, P. Allen, and D. G. Russell.** 1994. Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages. J. Immunol. **153:**2568–2578.